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Therapeutic activ vector

The present invention relates to retroviral vectors including a vector which undergoes promoter conversion (Procon vectors). The vector system is useful as a gene transfer vehicle for targeted gene therapy.

Background of the invention.

The use of retroviral vectors (RV) for gene therapy has received much attention and currently is the method of choice for the transferral of therapeutic genes in a variety of approved protocols both in the USA and in Europe (Kotani et al., 1994). However most of these protocols require that the infection of target cells with the RV carrying the therapeutic gene occurs in vitro, and successfully infected cells are then returned to the affected individual (Rosenberg et al., 1992; for a review see Anderson, 1992). Such ex vivo gene therapy protocols are ideal for correction of medical conditions in which the target cell population can be easily isolated (e.g. lymphocytes). Additionally the ex vivo infection of target cells allows the administration of large quantities of concentrated virus which can be rigorously safety tested before use.

Unfortunately, only a fraction of the possible applications for gene therapy involve target cells that can be easily isolat d, cultured and then reintroduced. Additionally, the complex t chnology and associat d high costs of ex vivo

gen therapy effectively preclude its disseminated use world-wide. Future facile and cost-effective gene therapy will require an in vivo approach in which the viral vector, or cells producing the viral vector, are directly administered to the patient in the form of an injection or simple implantation of RV producing cells.

This kind of in vivo approach, of course, introduces a variety of new problems. First of all, and above all, safety considerations have to be addressed. Virus will be produced, possibly from an implantation of virus producing cells, and there will be no opportunity to precheck the produced virus. It is important to be aware of the finite risk involved in the use of such systems, as well as trying to produce new systems that minimize this risk.

The essentially random integration of the proviral form of the retroviral genome into the genome of the infected cell led to the identification of a number of cellular proto-oncogenes by virtue of their insertional activation (Varmus, 1988). The possibility that a similar mechanism may cause cancers in patients treated with RVs carrying therapeutic genes intended to treat other pre-existent medical conditions, has posed a recurring ethical problem. Most researchers would agree that the probability of a replication defective RV, such as all those currently used, integrating into or near a cellular gene involving in controlling cell proliferation is vanishingly small. However it is generally also assumed that the explosive expansion of a population of replication competent retrovirus from a single infection event, will eventually provide enough integration events to make such a phenotypic integration a very real possibility.

Retroviral vector systems are optimized to minimize the chance of replication competent virus being present. However it has been well documented that recombination

events between components of th RV system can lead to the gen ration of potentially pathogenic replication competent virus and a number of generations of vector systems have been constructed to minimize this risk of recombination (reviewed in Salmons and Günzburg, 1993). However little is known about the finite probability of these events. Since it will never be possible to reduce the risk of this system to zero, an informed risk-benefit decision will always have to be taken. Thus it becomes very important to empirically determine the chance of (1) insertional disruption or activation of single genes by retrovirus integration and (2) the risk of generation of replication competent virus by recombination in current generations of packaging cell lines. A detailed examination of the mechanism by which these events occur will also allow the construction of new types of system designed to limit these events.

A further consideration when considering the use of <u>in vivo</u> gene therapy, both from a safety stand point and from a purely practical stand point, is the targeting of RVs. It is clear that therapeutic genes carried by vectors should not be indiscriminately expressed in all tissues and cells, but rather only in the requisite target cell. This is especially important if the genes to be transferred are toxin genes aimed at ablating specific tumour cells. Ablation of other, nontarget cells would obviously be very undesirable. Targeting of the expression of carried therapeutic genes can be achieved by a variety of means.

Retroviral vector systems consist of two components (Fig. 1):

1) the retroviral vector itself is a modified retrovirus (vector plasmid) in which the genes encoding for the viral proteins have been replaced by therapeutic genes and marker genes to be transferred to the target cell. Since the replacem nt of the genes encoding for the vir l proteins

effectively cripples th virus it must b rescu d by the second component in the system which provides the mi sing viral prot ins to the modified retrovirus.

The second component is:

2) a cell line that produces large quantities of the viral proteins, however lacks the ability to produce replication competent virus. This cell line is known as the packaging cell line and consists of a cell line transfected with a second plasmid carrying the genes enabling the modified retroviral vector to be packaged.

To generate the packaged vector, the vector plasmid is transfected into the packaging cell line. Under these conditions the modified retroviral genome including the inserted therapeutic and marker genes is transcribed from the vector plasmid and packaged into the modified retroviral particles (recombinant viral particles). A cell infected with such a recombinant viral particle cannot produce new vector virus since no viral proteins are present in these cells. However the vector carrying the therapeutic and marker genes is present and these can now be expressed in the infected cell.

It is an object of the present invention to provide a novel retroviral vector which can be used as a safe gene transfer vehicle for targeted gene therapy with a reduced probability to undergo genetic recombination with the packaging vector and a higher selectivity for site specific recombination with cellular sequences.

To achieve the foregoing and other objects, the invention provides a retroviral vector undergoing promoter conversion comprising a 5' LTR region of the tructure U3-R-U5;
one or more sequences selected from coding and non-coding

sequ nces; and a 3' LTR region comprising a completely or partially delet d U3 r gion wherein said delet d U3 region is replaced by a polylinker sequence, followed by the R and U5 region.

Said polylinker sequence carries at least one unique restriction site and contains preferably at least one insertion of a heterologous DNA fragment. Said heterologous DNA fragment is preferably selected of regulatory elements and promoters, preferably being target cell specific in their expression.

Further objects, features and advantages will be apparent from the following description of preferred embodiments of the invention.

The target cell specific regulatory elements and promoters are selected from one or more elements of the group consisting of Whey Acidic Protein (WAP), Mouse Mammary Tumour Virus (MMTV), ß-lactoglobulin and casein specific regulatory elements and promoters, pancreas specific regulatory elements and promoters including carbonic anhydrase II and ß-glucokinase regulatory elements and promoters , lymphocyte specific regulatory elements and promoters including immunoglobulin and MMTV lymphocytic specific regulatory elements and promoters and MMTV specific regulatory elements and promoters conferring responsiveness to glucocorticoid hormones or directing expression to the mammary gland. Said regulatory elements and promoters regulate preferably the expression of at least one of the coding sequences of said retroviral vector. The LTR regions are selected from at least one element of the group consisting of LTRs of Murine Leukaemia Virus (MLV), Mouse Mammary Tumour Virus (MMTV), Murine Sarcoma Virus (MSV), Simian Immunodeficiency Virus (SIV), Human Immunodeficiency Virus (HIV), Human T-cell Leukaemia Virus (HTLV), Feline Immunodefici ncy Virus (FIV), Feline Leukaemia Virus

(FELV), Bovine L ukaemia Virus (BLV) and Mason-Pfizer-Monkey virus (MPMV).

The retroviral vector is preferably a BAG vector (Price et al., 1987).

The coding sequence is preferably selected from one or more elements of the group consisting of marker genes, therapeutic genes, antiviral genes, antitumour genes, cytokine genes.

Said marker and therapeutic genes are preferably selected from one or more elements of the group consisting of B-galactosidase gene, neomycin gene, Herpes Simplex Virus, thymidine kinase gene, puromycin gene, cytosine deaminase gene, hygromycin gene, secreted alkaline phosphatase gene, guanine phosphoribosyl transferase (gpt) gene, alcohol dehydrogenase gene and hypoxanthine phosphoribosyl transferase (HPRT) gene.

According to the invention at least one retroviral sequence encoding for a rectoviral protein involved in integration of retroviruses is altered or at least partially deleted.

Said heterologous DNA fragment is preferably homologous to one or more cellular sequences. The regulatory elements and promoters are preferably regulatable by transacting molecules.

In a further embodiment of the invention a retroviral vector system is provided comprising a retroviral vector as described above as a first component and a packaging cell line harbouring at least one retroviral or recombinant retroviral construct coding for proteins required for said retroviral vector to be packaged.

The packaging cell lin harbours retroviral or recombinant retroviral constructs coding for those retroviral proteins which are not encoded in said retrovir l vector. The packaging cell line is preferably selected from an element of the group consisting of ψ2, ψ-Crypt, ψ-AM, GP+E-86, PA317 and GP+envAM-12.

After replicating the retroviral vector of the invention as described above in a retroviral vector system as described above, a retroviral provirus is provided wherein said polylinker and any sequences inserted in said polylinker in the 3'LTR become duplicated during the process of reverse transcription in the infected target cell and appear in the 5'LTR as well as in the 3'LTR of the resulting provirus.

The invention includes also mRNA of a retroviral provirus according to the invention and any RNA resulting from a retroviral vector according to the invention.

A further embodiment of the invention provides non-therapeutical method for introducing homologous and/or heterologous nucleotide sequences into human or animal cells in vitro and in vivo comprising transfecting a packaging cell line of a retroviral vector system according to the invention with a retroviral vector according to the invention and infecting a target cell population with recombinant retroviruses produced by the packaging cell line. The nucleotide sequences are selected from one or more elements of the group consisting of genes or parts of genes encoding for proteins, regulatory sequences and promoters.

The retroviral vector, the retroviral vector system and the retroviral provirus as well as RNA thereof is used for producing a pharmaceutical composition for gene therapy in mammals including humans. Furthermore, they are used for targeted int gration in homologous cellular sequ nc s.

Promoter conversion

The present invention uses the principle of promoter conversion typical for retroviruses.

The retroviral genome consists of an RNA molecule with the structure R-U5-gag-pol-env-U3-R (Fig. 2). During the process of reverse transcription, the U5 region is duplicated and placed at the right hand end of the generated DNA molecule, whilst the U3 region is duplicated and placed at the left hand end of the generated DNA molecule (Fig. 2). The resulting structure U3-R-U5 is called LTR (Long Terminal Repeat) and is thus identical and repeated at both ends of the DNA structure or provirus. The U3 region at the left hand end of the provirus harbours the promoter (see below). This promoter drives the synthesis of an RNA transcript initiating at the boundary between the left hand U3 and R regions and terminating at the boundary between the right hand R and U5 region (Fig. 2). This RNA is packaged into retroviral particles and transported into the target cell to be infected. In the target cell the RNA genome is again reverse transcribed as described above.

According to the invention a retroviral vector is constructed in which the righthand U3 region is altered (Fig. 3), but the normal lefthand U3 structure is maintained (Fig. 3); the vector can be normally transcribed into RNA utilizing the normal retroviral promoter located within the left hand U3 region (Fig. 3). However the generated RNA will only contain the altered righthand U3 structure. In the infected target cell, after reverse transcription, this altered U3 structure will be placed at both ends of the retroviral structure (Fig. 3).

If the altered region carries a polylinker (see b low) instead of th U3 region then any promoter, including those

directing tissue sp cific expression such as the WAP promoter (see b low) can be easily inserted. This prom t r will then be utilized exclusively in the target cell for expression of linked genes carried by the retroviral vector. Alternatively or additionally DNA segments homologous to one or more celluar sequences can be inserted into the polylinker for the purposes of gene targeting (see below).

According to the invention the term "polylinker" is used for a short stretch of artificially synthesized DNA which carries a number of unique restriction sites allowing the easy insertion of any promoter or DNA segment. The term "heterologous" is used for any combination of DNA sequences that is not normally found intimately associated in nature.

Gene expression is regulated by promoters. In the absence of promoter function a gene will not be expressed. The normal MLV retroviral promoter is fairly unselective in that it is active in most cell types. However a number of promoters exist that show activity only in very specific cell types. Such tissue-specific promoters will be the ideal candidates for the regulation of gene expression in retroviral vectors, limiting expression of the therapeutic genes to specific target cells.

In the packaging cell line the expression of the retroviral vector is regulated by the normal unselective retroviral promoter (Fig. 3). However as soon as the vector enters the target cell promoter conversion occurs, and the therapeutic genes are expressed from a tissue specific promoter of choice introduced into the polylinker (Fig. 3). Not only can virtually any tissue specific promoter be included in the system, providing for the selective targeting of a wide variety of different cell types, but additionally, following the conversion event, the structur and properties of the retroviral vector no longer resembl s

that f a virus. This, of course, has extr mely important consequences from a saf ty point of view, sinc ordinary or state of the art retroviral vectors r adily undergo genetic recombination with the packaging vector to produce potentially pathogenic viruses. Promoter conversion (Procon) vectors do not resemble retroviruses because they no longer carry U3 retroviral promoters after conversion thus reducing the possibility of genetic recombination.

The retroviral promoter structure is termed LTR. LTRs carry signals that allow them to jump in and out of the genome of the target cell. Such jumping transposable elements can also contribute to pathogenic changes. Procon vectors can carry modified LTRs that no longer carry the signals required for jumping. Again this increases the potential safety of these vector systems.

Gene Targeting

According to another aspect of the present invention the retroviral vector is used for targeted integration into the target cell. The integration of the proviral DNA version of the retroviral genome into the target cell is a major advantage to the use of retroviruses as vectors when compared to other viruses such as adenoviruses, since it allows long term stable expression of transferred genes. However the random nature of this integration event also poses a major disadvantage to RV use since it raises the possibility of insertional (in)activation of cellular tumour suppressor genes or proto-oncogenes and thus tumour induction.

Homologous recombination has been successfully used to targ t the int gration of transfected or microinj cted DNA to specific DNA loci and is routinely used in the

construction of "knock-out" transg nic mice or animals (reviewed in Capecchi, 1989; Bradley et al., 1992; Morrow and Kucherl pati, 1993). Unfortunately the efficiency f DNA transfer by such purely physical methods is extremely low. In contrast retroviral mediated gene transfer is very efficient, almost 100% of a population of cells being infectable. A combination of retroviral gene transfer with homologous recombination should allow the construction of an ideal system for locus targeted integration.

We have investigated the feasibility of introducing long homologous pieces of DNA into RVs in different locations to promote integration by homologous recombination. Both gene conversion and homologous recombination have been evaluated. Using a cell line carrying a single copy of the HSV-tk gene as a target we have been able to disrupt the target at frequencies 15 fold higher than previously reported by others (Ellis and Bernstein, 1989). Cloning out of the recombined fragments of DNA has revealed the presence of both target tk sequence and retroviral vector.

For targeted integration DNA segments homologous to cellular sequences are inserted into the polylinker of the Procon vectors. After infection of the target cell and reverse transcription, these sequences will appear at the 5' terminal end of the provirus. Terminal homologies have been shown to favour homologous recombination (Bradley, 1991) to isogenic cellular sequences. Infection of target cells which carry mutated versions of the homologous sequence should result in the recombination and thus repair of the mutated sequence. Either just the homologous sequences will recombine into the cellular genome, or the complete vector will be inserted. Not only has this vector class potential for use in gene repair, it can also be utilized to direct the integration of retroviral vectors carrying therapeutic genes to specific loci in the genome which are known not to harbour active genes. This will

r duce considerably the possiblity of insertional activation or inactivation as described above, and will thus contribute to the safety of the use of retroviral vectors.

The following examples will illustrate the invention further. These examples are however in no way intended to limit the scope of the present invention as obvious modifications will be apparent, and still other modifications and substitutions will be apparent to anyone skilled in the art.

The recombinant DNA methods employed in practicing the present invention are standard procedures, well known to those skilled in the art, and described in detail, for example, in Molecular Cloning, T. Maniatis, et al., Cold Spring Harbor Laboratory, (1982) and B. Perbal, A Practical Guide to Molecular Cloning, John Wiley & Sons (1984).

Example 1

Principle for the Construction of Promoter Vector for targeted gene expression

In the murine leukemia virus (MLV) retroviral vector known as BAG (Price et al., 1987) the ß-galactosidase gene is driven by the promiscuous (i.e. non-tissue specific) MLV promoter in the U3 region of the LTR (Fig. 3). According to the present invention a derivative of the BAG vector has been constructed in which the MLV promoter (U3) located within the 3'LTR (Fig. 3) has been deleted and replaced with a polylinker, said polylinker allowing the facile introduction of heterologous promoters. The BAG vector lacking the U3 is expressed from the MLV promoter (U3) within the 5'LTR when introduced into a packaging cell line. As a result of the rearrangements occurring in the retroviral genome during its life cycle, following infection of its target cell, the polylinker will be duplicated at both ends of the retroviral genome as described above. Thereby a retroviral vector can be constructed in which the expression of the ß-galactosidase gene of BAG will be controlled by the polylinker or any promoter inserted into the polylinker in the target cell (Fig. 3).

According to the principle set forth above the following specific promoters have been inserted into the polylinker region of the modified BAG vector:

Several subregions of the Mouse Mammary Tumour Virus (MMTV) promoter including a region that confers responsiveness to glucocorticoid hormones and a region containing an element that directs expression to the mammary gland.

The Whey Acidic Protein (WAP) promoter. This promoter controls the expression of WAP so that it is only produced

in the mammary glands of pregnant and lactating rodents.

The control of the ß-galactosidase gene expression by promoters inserted into the polylinker has been validated by infection studies of the constructed MMTV and WAP retroviral vectors of primary mammary gland cells (Fig. 4), demonstrating expression of both the WAP-BAG and MMTV-BAG retroviral vectors.

The MMTV and WAP Procon vectors have been transfected into the packaging cell line GP+E86 (Markowitz et al., 1988). After selection for neomycin resistance, which is encoded by the vector, stable populations and clones of recombinant Procon virus producing cells were obtained. Virus containing supernatant from these populations was used to infect a mouse mammary cell line EF43 (Günzburg et al., 1988) as well as a mouse fibroblast cell line (Jainchill et al., 1969). Four days after infection the target cells were lysed and quantitative ß-galactosidase assay revealed no expression in either cell type infected by the WAP carrying Procon vectors and good expression in both cell types from the MMTV carrying Procon vector (Fig. 5). This result is in accordance with the WAP promoter only functioning in vivo during late pregnancy and lactation and not in most simple in vitro cell culture systems. To investigate whether the WAP carrying Procon vectors would be active in a complex primary mammary derived cell culture system, primary organoids were prepared and cultured from 8-10 day pregnant mice and infected with the supernatant from the same stably transfected population of transfected cell lines. Both Procon vectors carrying the WAP and the MMTV promoter fragments were active in these primary cell lines as demonstrated by ß-galactosidase activity (Fig.5).

To investigate whether the WAP and MMTV carrying Procon vect rs were active in vivo and whether the expression of ß-galactosidase was limited to the mammary gland in vivo,

recombinant Procon virus containing medium was injected *in situ* into the mammary glands or skin of 8-10 day pregnant mice. Five days later the mice wer sacrificed, cell extracts prepared and a ß-galactosidase assay performed. Both the WAP and MMTV fragment carrying Procon vectors were expressed only in the pregnant mammary gland and not in the skin (Fig. 6). Thus *in vivo* the regulatory elements from both promoters limit expression to the mammary gland whereas *in vitro* the regulatory elements from the WAP promoter retain their strict tissue specificity but those of MMTV do not.

These Procon vectors carrying tissues specific promoters and regulatory elements will be useful for directing the expression of therapeutic genes to predefined cell types, tissues and organs. Potential therapeutic genes include mellitin, which has anti-HIV and anti-tumour effects, and genes which prime cells for death including the thymidine kinase, guanine phosphoribosytransferase and cytosine deaminase genes.

Example 2

Construction of Procon Vectors for targeted integration

Using the same BAG vector described in Example 1 above, a retroviral vector can be constructed in which a DNA sequence with homology to a cellular sequence can be inserted into the LTR. The resulting vector can be used to target the integration of either the homologous sequence inserted into the vector or the whole or part of the vectors into the homologous sequence present in the host cell genome.

According to the principle s t forth above, a fragm nt of the thymidine kinase (tk) gene of herp s simplex virus (HSV) has been inserted into the polylinker region of the modified BAG vector:

A cell line has also been established that has no functional copy of the mammalian tk gene and instead carries one copy of the HSV-tk gene. This cell line has been infected with the tk carrying BAG vector and cells that have undergone disruption of the HSV-tk gene have been selected (Fig. 7).

The above examples have illustrated the principles and consequences of the promoter conversion vectors provided by the present invention.

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Therapeutic active vector

C L A I M S

1. A retroviral vector undergoing promoter conversion comprising a 5' LTR region of the structure U3-R-U5;
one or more sequences selected from coding and non-coding sequences; and
a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence, followed by the R and U5 region.

2. A retroviral vector according to claim 1, wherein said polylinker sequence carries at least one unique restriction site.

3. A retroviral vector according to claim 2, wherein said polylinker sequence contains at least one insertion of a heterologous DNA fragment.

4. A retroviral vector according to claim 3, wherein said heterologous DNA fragment is selected from one or more elements of the group consisting of regulatory el ments and promoters.

5. A retroviral vector according to claim 4, wh rein said regulatory elements and promoters are target cell specific in their expression.

6. A retroviral vector according to claim 5, wherein said target cell specific regulatory elements and promoters are selected from one or more elements of the group consisting of WAP, MMTV, ß-lactoglobulin and casein specific regulatory elements and promoters, pancreas specific regulatory elements and promoters including carbonic anhydrase II and ß-glucokinase regulatory elements and promoters , lymphocyte specific regulatory elements and promoters including immunoglobulin and MMTV lymphocytic specific regulatory elements and promoters and MMTV specific regulatory elements and promoters conferring responsiveness to glucocorticoid hormones or directing expression to the mammary gland.

7. A retroviral vector according to anyone of claims 1 to 6, wherein said regulatory elements and promoters regulate the expression of at least one of the coding sequences of said retroviral vector.

8. A retroviral vector according to anyone of claims 1 to 7, wherein said LTR regions are selected from at least one element of the group consisting of LTRs of MLV, MMTV, MSV, SIV, HIV, HTLV, FIV, FeLV, BLV and MPMV.

9. A retroviral vector according to anyone of claims 1 to 8, wherein said retroviral vector is a BAG vector.

10. A retroviral vector according to anyone of claims 1 to 9, wherein said coding sequence is selected from one or more elements of the group consisting of marker genes, therapeutic genes, antiviral genes, antitumour genes, cytokine

genes.

11. A retroviral vector according to claim 10, wherein said marker or therapeutic gene is selected from one or more elements of the group consisting of ß-galactosidase gene and neomycin gene, Herpes Simplex Virus, thymidine kinase gene, puromycin gene, cytosine deaminase gene, hygromycin gene, secreted alkaline phosphatase gene, guanine phosphoribosyl transferase (gpt) gene, alcohol dehydrogenase gene and hypoxanthine phosphoribosyl transferase (HPRT) gene.

12. A retroviral vector according to anyone of claims 1 to 11, wherein at least one of said coding sequences for a retroviral protein is altered or at least partially deleted.

13. A retroviral vector according to anyone of claims 1 to 12, wherein retroviral sequences involved in integration of retroviruses are altered or at least partially deleted.

14. A retroviral vector according to anyone of claims 1 to 13, wherein said heterologous DNA fragment is homologous to one or more cellular sequences or a part thereof.

15. A retroviral vector according to anyone of claims 1 to 14, wherein said regulatory elements are regulatable by transacting molecules.

16. A retroviral vector system comprising a retroviral vector according to anyone of claims 1 to 15 as a first component; and
a packaging cell line harbouring at least one retroviral or recombinant retroviral construct coding for proteins

required for said retroviral vector to be packaged.

17. A retroviral vector system according to claim 16 wherein the packaging cell line harbours retroviral or recombinant retroviral constructs coding for those retroviral proteins which are not encoded in said retroviral vector according to anyone of claims 1 to 15.

18. A retroviral vector system according to claim 16 or 17 wherein the packaging cell line is selected from an element of the group consisting of ψ2, ψ-Crypt, ψ-AM, GP+E-86, PA317 and GP+envAM-12.

19. A non-therapeutical method for introducing homologous or heterologous nucleotide sequences into human or animal cells in vitro and in vivo comprising transfecting a packaging cell line of a retroviral vector system according to anyone of claims 16 to 18 with a retroviral vector according to anyone of claims 1 to 15, and infecting a target cell population with said recombinant retroviruses produced by the packaging cell line.

20. A non-therapeutical method according to claim 19, wherein the nucleotide sequences are selected from one or more elements of the group consisting of genes or parts of genes encoding for proteins, regulatory sequences anhd promoters.

21. A retroviral provirus produced by replicating the retroviral vector of anyone of claims 1 to 15 in a retroviral vector system according to anyone of claims 16 to 18 wherein said polylinker and any sequences inserted in said polylinker in the 3'LTR become duplicated during the process of reverse transcription in the infected target cell and appear in the 5'LTR as well as in the 3'LTR of th

resulting provirus.

22. Use of a retroviral vector according to anyone of claims 1 to 15 for producing a pharmaceutical composition for gene therapy in mammals including humans.

23. Use of a retroviral vector system according to anyone of claims 16 to 18 for producing a pharmaceutical composition for gene therapy in mammals including humans.

24. Use of a retroviral provirus according to claim 23 for producing a phamaceutical composition for gene therapy in mammals including humans.

25. Use of a retroviral vector according to anyone of claims 1 to 15 for targeted integration in said homologous cellular sequences.

26. Use of a retroviral vector system according to anyone of claims 16 to 18 for targeted integration in said homologous cellular sequences.

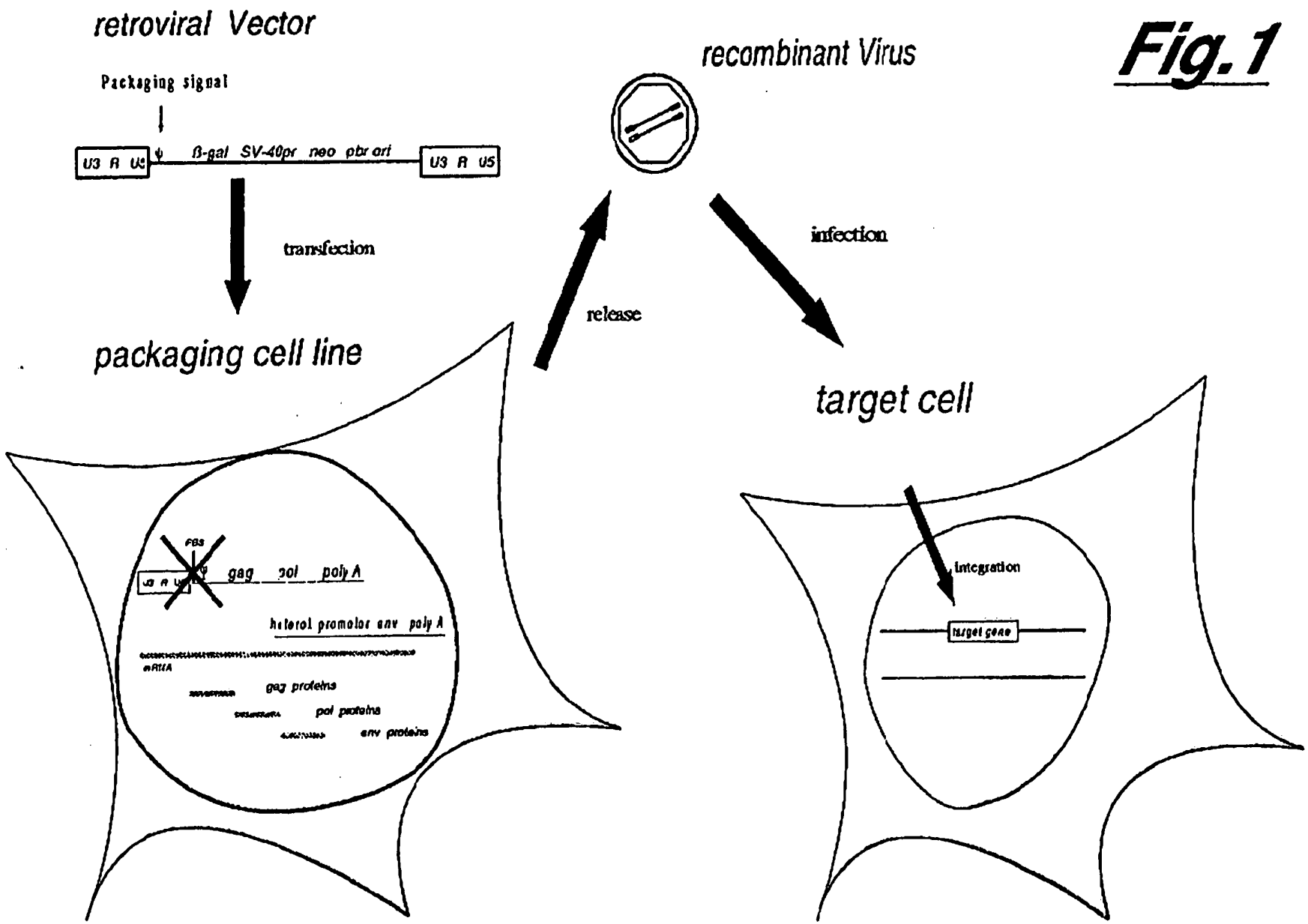
27. Use of a retroviral provirus according to claim 21 for targeted integration in said homologous cellular sequences.

28. mRNA of a retroviral provirus according to claim 21.

29. RNA of a retroviral vector according to anyone of claims 1 to 15.

SUMMARY OF THE INVENTION

The present invention relates to a retroviral vector undergoing promoter conversion comprising a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence, followed by the R and U5 region.
Said retroviral vector undergoes promoter conversion and is useful as a gene transfer vehicle for targeted gene therapy.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ß-gal SV-40pr neo pbr ori
U3 R U5
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recombinant Virus
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target cell
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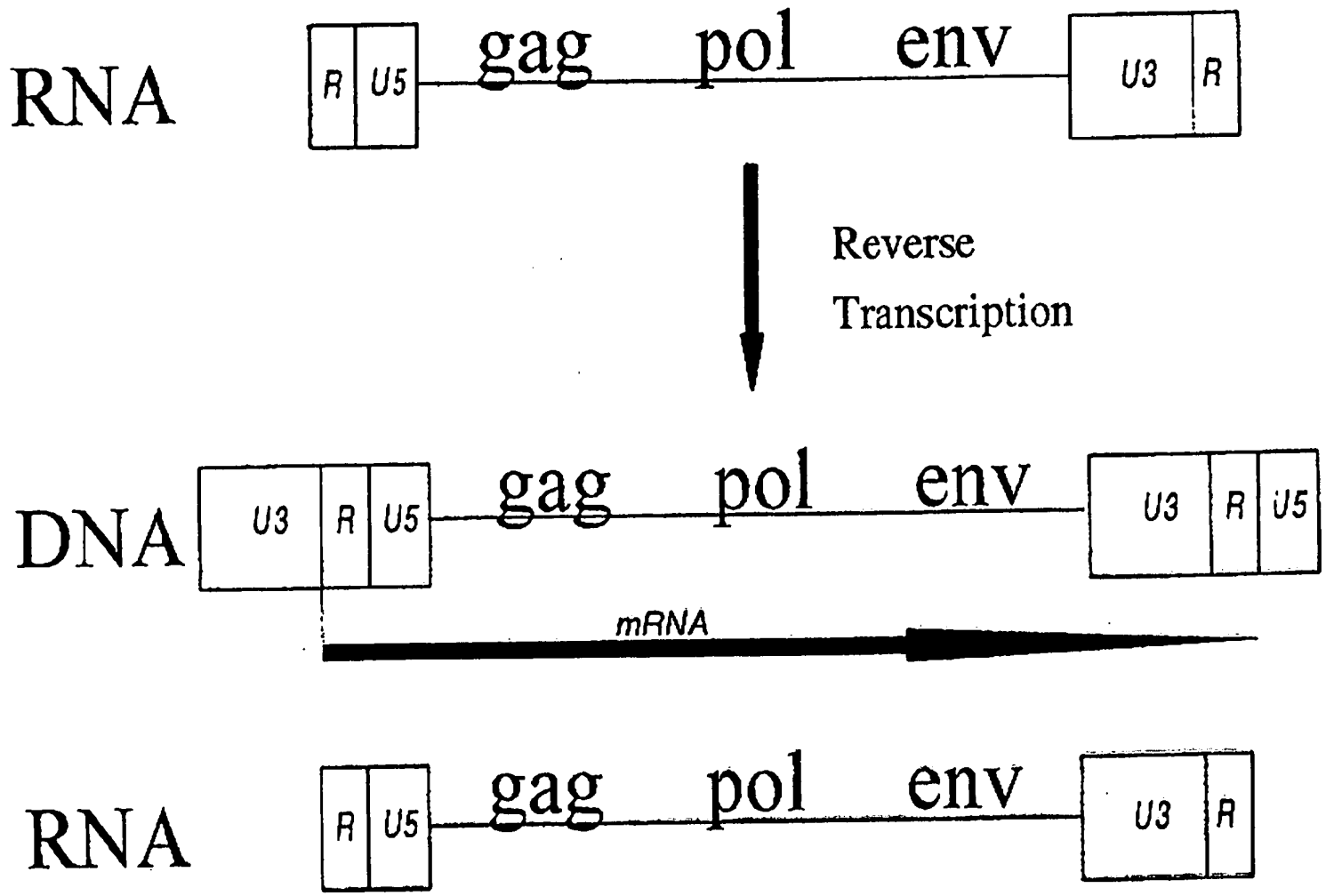
gag proteins
pol proteins
env proteins
integration
target gene

Fig. 1

Fig. 2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RNA
R
U5
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pol
env
U3
R

Fig. 3

Construction of a U3 minus BAG-vector (MLV)

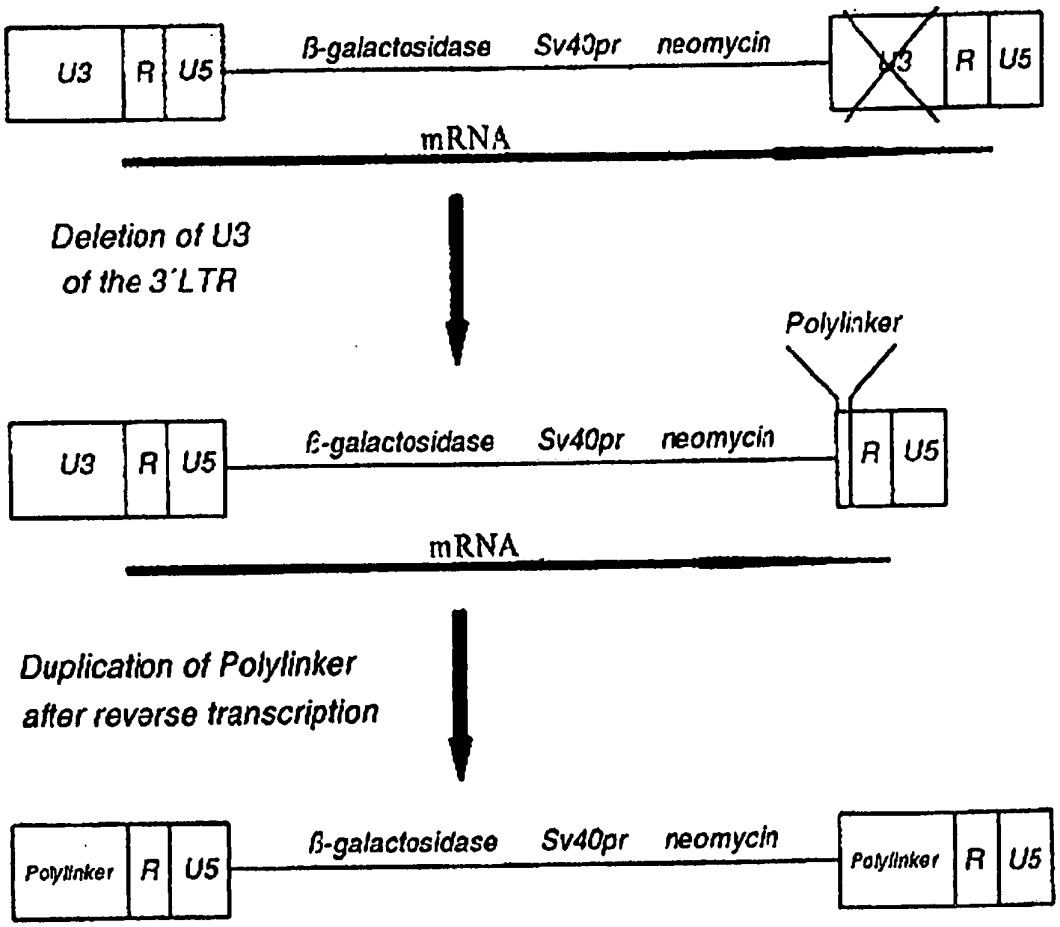

Fig.4

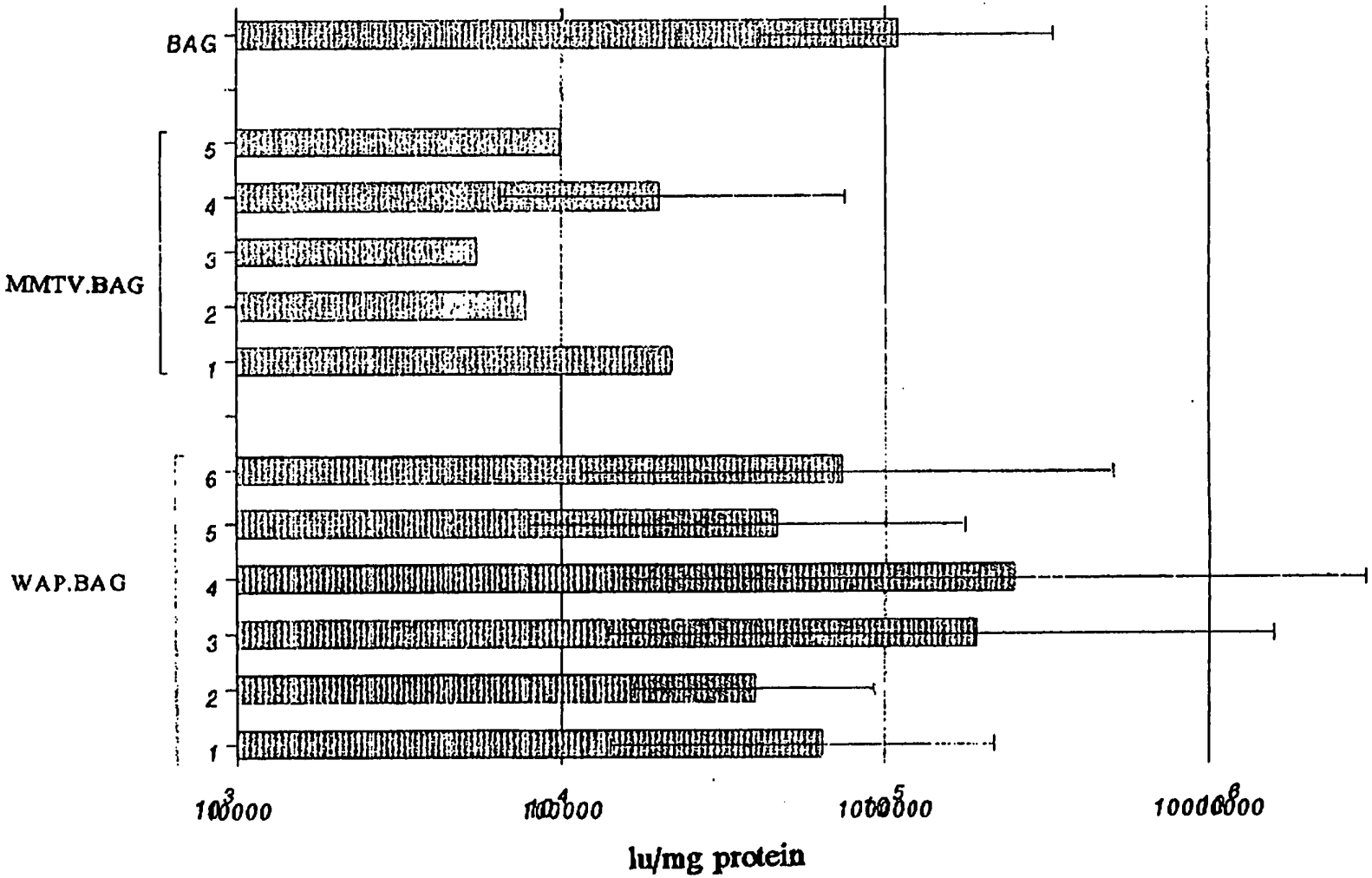
BAG
MMTV.BAG
5
4
3
2
1
WAP.BAG
6
5
4
3
2
1
10000
100000
1000000
10000000
lu/mg protein

Fig.5

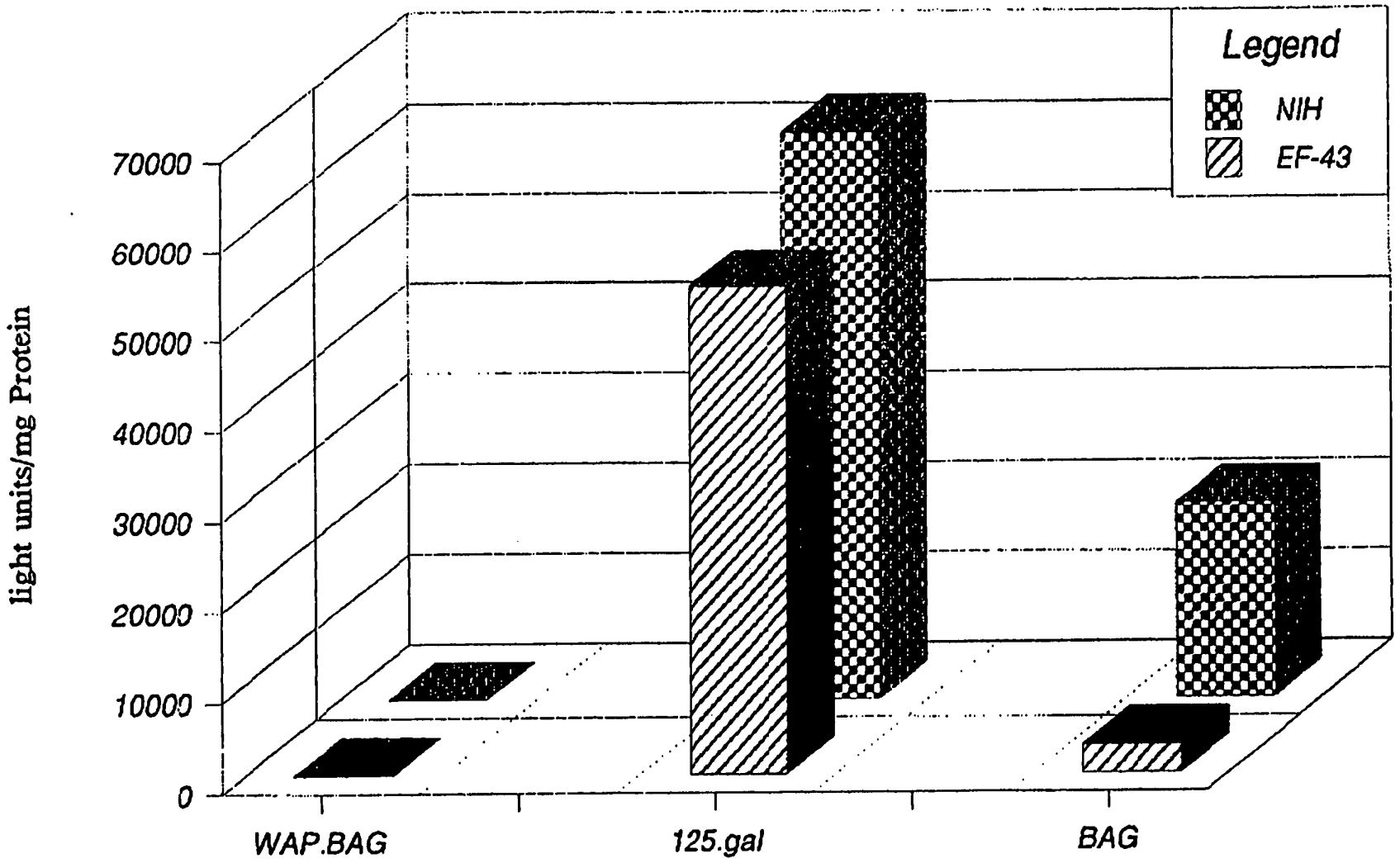
light units/mg Protein
70000
60000
50000
40000
30000
20000
10000
0
WAP.BAG
125.gal
BAG
Legend
NIH
EF-43

Fig6. pat

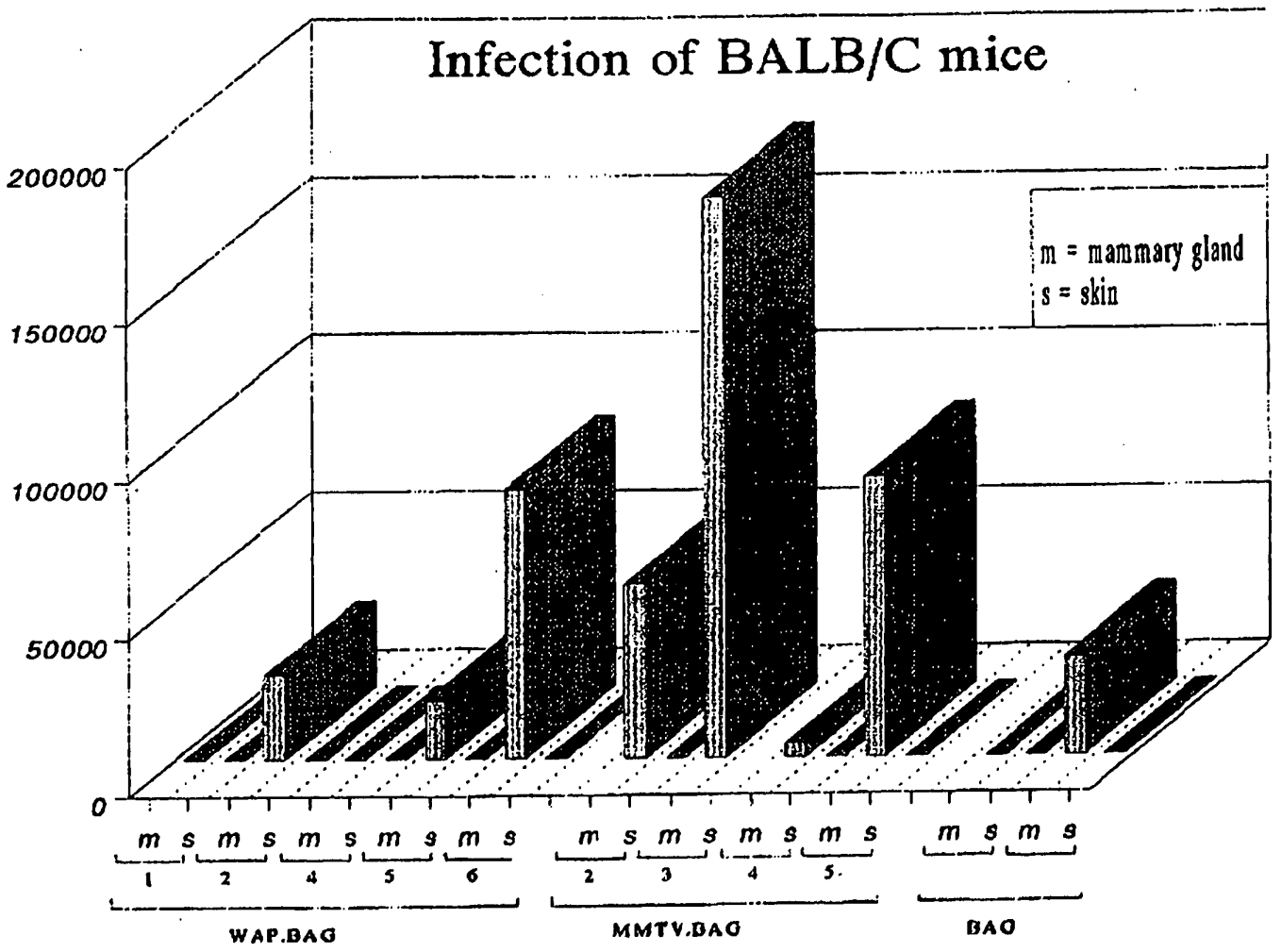
Infection of BALB/C mice
200000
150000
100000
50000
0
m = mammary gland
s = skin
m s m s m s m s m s
1 2 4 5 6
WAP.BAG
m s m s m s m s
2 3 4 5
MMTV.BAG
m s m s
BAG

Fig. 7